CALLUS INDUCTION AND DEVELOPMENT OF SUSPENSION CELL CULTURE OF MALAYSIAN UPLAND RICE CULTIVAR *PANDERAS*

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To my beloved family, friends and lecturers

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ABSTRACT

The aim of the study is to evaluate the effect of amino acid (tryptophan and glutamine) on the callus induction and to develop suspension cell culture protocol for Malaysian upland rice, *Panderas* cultivar. The research revealed that callus induction varied depend on amino acid tested. In callus induction study, dehusked *Panderas* mature seeds were placed on MSB_5 medium supplemented with 3mg/L 2,4-D and 2mg/L NAA with four different concentrations of tryptophan and glutamine separately. After three weeks in culture, inclusion of tryptophan showed positive effect on percentage of callus induction and fresh weight. It was observed that the optimum concentration of tryptophan was 25mg/L with 96.67% of callus induction and 73.33mg of average fresh weight. At week six, the average fresh weight of callus induced on MSB_5 media supplemented with 25mg/L tryptophan was increased to three fold (215mg). Treatment without amino acids (control) showed similar callus percentage (96.67%) and three times increased in fresh weight after six weeks. However addition of 50mg/L glutamine alone showed lower percentage of callus induction and fresh weight compare to tryptophan and control treatment. The callus proliferation resulted in yellowish colour and nodular appearance showing embryogenic potential. The embryogenic callus was then immersed in 1% Evans blue to validate the viability of cells. Five weeks old potential embryogenic callus was then selected to initiate suspension cell culture. The N_6 liquid medium supplemented with 3mg/L 2,4-D, 1mg/L kinetin and 0.005% pectinase resulted in higher fresh weight (1.84g) of suspension cells compared to without pectinase on day ten of incubation. This study concludes that tryptophan and glutamine did not show significant response on percentage of callus induction and fresh weight. Besides that inclusion of pectinase in suspension cell culture may increase fresh weight of suspension cell.

ABSTRAK

Tujuan kajian ini dijalankan adalah untuk mengenal pasti keberkesanan asid amino (triptofan dan glutamin) dalam penginduksian kalus embriogeni dan mengkaji pembangunan kultur sel ampaian bagi padi bukit Malaysia kultivar Panderas. Untuk penginduksian kalus embriogeni, sampel biji benih yang telah dibuang kulitnya diletakkan di atas permukaan media MSB_5 yang mengandungi 3mg/L 2,4-D dan 2mg/L NAA serta ditambah dengan empat kepekatan berbeza triptofan and glutamin secara berasingan. Selepas tiga minggu dikultur, penambahan triptofan menunjukkan kesan positif. Berdasarkan pemerhatian, media penambahan 25mg/L triptofan dapat menginduksikan kalus sehingga 96.67% dan purata berat basah sebanyak 73.33mg. Purata berat basah kalus yang dikultur di atas media ini meningkat sebanyak tiga kali ganda selepas enam minggu. Media tanpa asid amino (kawalan) mengekalkan peratusan penginduksian kalus (96.67%) tetapi berat basah meningkat sebanyak tiga kali ganda pada minggu keenam. Bagaimanapun, penambahan 50mg/L glutamin sahaja menunjukkan peratus pertumbuhan kalus dan berat basah yang rendah berbanding dengan triptofan dan kawalan. Morfologi kalus embriogeni yang diperolehi menunjukkan warna kuning dan berbentuk nodular. Penentuan kebolehidupan sel diperolehi dengan merendamkan kalus ke dalam 1% Evans biru. Seterusnya, kalus embriogeni yang berumur lima minggu dipilih untuk membangunkan kultur sel ampaian. Media cecair N_6 yang ditambah dengan 3mg/L 2,4-D, 1mg/L kinetin dan 0.005% pektinase menghasilkan berat basah sel ampaian yang tinggi (1.84g) pada hari ke-sepuluh pengeraman. Kajian mendapati triptofan dan glutamin tidak menunjukkan perbezaan ketara terhadap peratus pertumbuhan kalus dan berat basah. Selain daripada itu, penambahan pektinase di dalam kultur sel ampaian dapat meningkatkan berat basah sel ampaian.